

Detection and Measurement of Decomposition in Endrin Standards

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A study at elevated and room temperatures shows that the percentage of undecomposed endrin in a standard may be determined readily from the infrared spectra in potassium bromide using the absorbance ratio of the peaks at 1600 and 1750 cm^{-1} . Since the use of this ratio eliminates con-

centration and instrument variables, calibration data are given which can be used for any instrument. Endrin standards should be evaluated upon receipt and stored at low temperatures in opaque containers.

Endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,endo*-5,8-dimethanonaphthalene) has wide usage as an insecticide for food crops. It is decomposed by heat and absorption of light (Mitchell, 1961; Phillips *et al.*, 1962; Roburn, 1963) to form isomeric ketone, aldehyde, and other decomposition products which are less toxic than endrin itself (Rosen *et al.*, 1966). As a consequence, some endrin standards are stabilized by addition of small amounts of organic stabilizers such as hexamethylenetetramine.

Endrin residues in food and other products are usually determined by gas-liquid chromatography using electron-capture detection after separation by extraction and column chromatography (Mills *et al.*, 1963). Since such residues are usually very small, the actual purity of the endrin standard used for comparison and calculation is very important. One method for the determination of the amount of endrin in pesticidal formulations (Shell Chemical Co., 1958) compares the absorbance of the epoxide peak at 11.76 microns in carbon disulfide solution with that of a reference standard. Analytical standard endrin (Shell Chemical Co.) is prepared by multiple recrystallizations until no further spectrum changes are observed in the carbonyl region of the infrared. This is referred to as 99% rather than 100% weight endrin as academic acknowledgment that minute amounts of other materials may be present (Glasser, 1968).

This paper reports a study of the thermal decomposition of endrin at elevated temperatures by infrared absorbance in potassium bromide and suggests a simple method for the determination of decomposition in endrin standards which is applicable to standards assumed to be relatively free of impurities such as isodrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,endo*-5,8-dimethanonaphthalene) and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene). The method determines the per cent of undecomposed endrin by measuring the absorbance ratio of the undecomposed endrin and the ketone decomposition

product. These percentages are relative rather than absolute, since there are other decomposition products. However, for standards which contain few impurities and in which there is only slight decomposition (up to approximately 10% or the appearance of the first infrared evidence of the aldehyde decomposition product), the relative values should be very close to the absolute values. Since the method does not detect the presence of impurities, it cannot be used for the determination of absolute purity. This work also shows the need for low temperature storage of standards in opaque containers.

EXPERIMENTAL

Apparatus and Reagents. Infrared spectrometers, Perkin Elmer Model 337, Beckman Model IR-5 plus others listed in Table II.

Gas-liquid chromatographs, Barber-Colman Model 10 with electron-capture detector and a 1/4-inch by 6-foot column of 10% DC-200 oil on 100/120-mesh Chrom Q using nitrogen at 20-p.s.i. pressure. The injection port was held at 228° C., the column at 210° C., and the detector at 195° C. Also a Barber-Colman Model 5000 with an electron-capture detector and a 4-mm. by 3-foot column of 3% XE-60 on 100/120-mesh Chrom Q using nitrogen at a flow rate of 50 ml. per minute. The injection port was held at 232° C., the column at 220° C., and the detector at 270° C.

Potassium bromide, optical grade.

Ethyl acetate, Burdick and Jackson, distilled-in-glass grade.

Petroleum ether, Burdick and Jackson, distilled-in-glass grade.

ENDRIN STANDARDS AND SAMPLES. Standard A. A high purity standard received from the Food and Drug Administration, Washington, D.C., in 1963 and stored under refrigeration in amber bottle. Stated purity, better than 99%.

Standard B. High purity standard received from the Food and Drug Administration, Washington, in 1960 and stored at room temperature in Dallas.

Standard C. High purity commercial standard labeled 99.6% stored under unknown conditions in Brownsville, Tex. Age unknown.

Standard D. A second sample of endrin standard A which had been stored at room temperature at Food and Drug Administration in Washington and received by Dallas District in 1968.

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PROCEDURES

Thermal Decomposition Study. A 0.5% (w./w.) disk of endrin standard A in potassium bromide was heated in a constant temperature oven at 120° C. for 2-hour periods for a total of 72 hours and at longer times thereafter. The infrared spectrum was determined after each heat exposure and the disk was stored at room temperature between heating intervals. After extended periods at room temperature (overnight or over weekend) the spectrum was repeated before the disk was returned to the oven. No change was noted during any storage period. Other 0.5% (w./w.) disks were heated for 15 minutes at various temperatures and the infrared spectra determined. One 0.5% (w./w.) disk was stored in an incubator at 35° C. for several months, the infrared spectrum being determined periodically.

Replication Studies. The infrared spectra of 0.5% (w./w.) KBr disks of standards A, B, and C were determined on the instrument used for the thermal decomposition study and upon nine other infrared spectrometers. They were also determined periodically over a period of 14 days on the instrument used for the thermal decomposition study. A series of KBr disks of standard B of different concentrations was also run on the same instrument.

Gas-Liquid Chromatography. Samples of standards A, B, and C were dissolved in ethyl acetate and diluted with petroleum ether to the proper concentration levels. Using standard A for comparison before and after each run, chromatograms of standards B and C were obtained from two instruments.

Proposed Method for Determination of Decomposition. Prepare a KBr disk of the sample of approximately 0.5% (w./w.) and run the infrared spectrum. Use higher concentrations for samples which show little decomposition in order to increase the net absorbances and decrease the error of measurement. Measure net absorbances of the peaks at 1600 and 1750 cm^{-1} , using as a common base line a straight line drawn from the curve at 1550 cm^{-1} to the curve at 1850 cm^{-1} . Divide the net absorbance at 1600 cm^{-1} by the net absorbance at 1750 cm^{-1} to obtain the absorbance ratio. Read the per cent of undecomposed endrin from the calibration curve prepared by plotting absorbance ratio against per cent undecomposed endrin.

RESULTS

Thermal Decomposition. Portions of the infrared spectra of endrin standard A after various times of heating at 120° C. are shown in Figure 1. These peaks were selected, since the ketone decomposition product shows a strong absorbance at 1750 cm^{-1} while endrin has strong absorbance bands at 1600, 848, and 748 cm^{-1} . The variation of the net absorbances of these peaks with time of heating at 120° C. is shown in Figure 2. Since endrin standard A was known to contain some decomposition impurities, the absorption curve due to the ketone decomposition product at 1750 cm^{-1} was extrapolated to zero absorbance to obtain an estimated zero decomposition time. The absorption curves due to

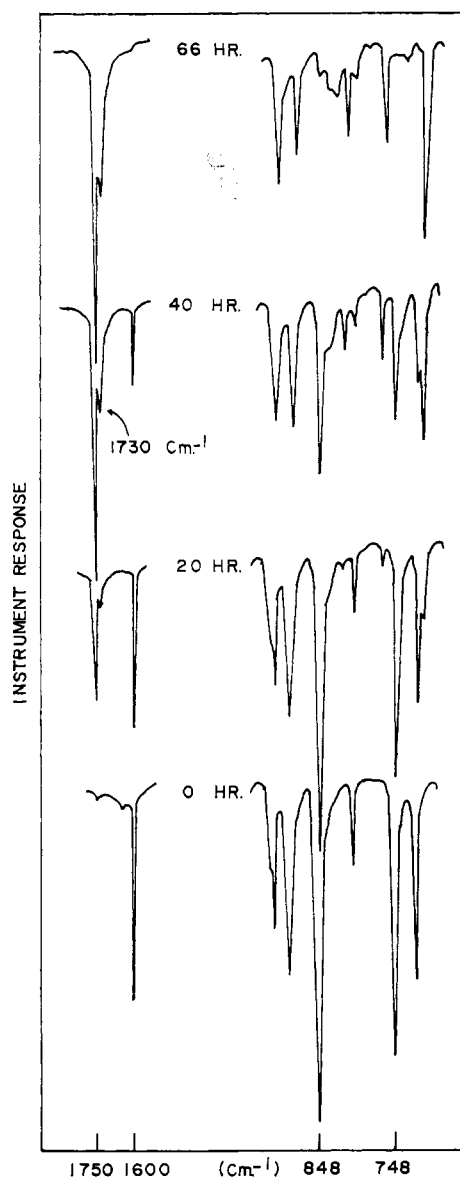


Figure 1. Infrared spectra of endrin after various periods of heating at 120° C.

endrin bands at 1600, 848, and 748 cm^{-1} were then extrapolated to this same time to determine their net absorbance at zero decomposition.

Apparently there are three distinct rates of decomposition based on time of heating at 120° C. The rate increases after approximately 11 hours and slows down after 38 hours. These times represent undecomposed endrin of approximately 91 and 32%, respectively. The endrin is apparently completely decomposed after 64 hours, since there is no change in net absorbance at 1600, 848, and 748 cm^{-1} after this time. The increase in rate after 11 hours may be related to the formation of the aldehyde isomer, since the peak at 1730 cm^{-1} (Figure 1) due to the aldehyde carbonyl first appears after this approximate time of heating. The variation of absorbance with time of heating at 120° C. for this peak is shown in Figure 2.

Net absorbance values at 10% intervals of the maxi-

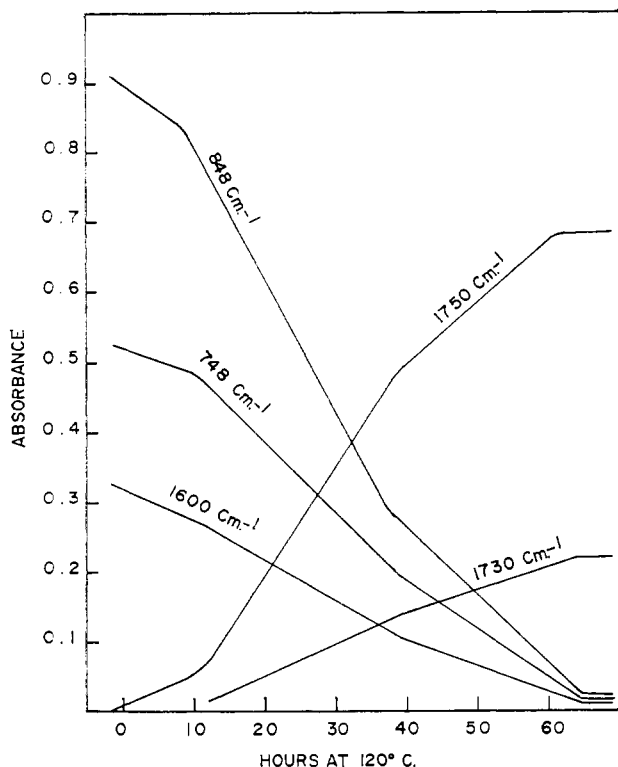


Figure 2. Variation of absorbance with time of heating at 120° C.

imum net absorbance for each peak were read from the absorbance curves in Figure 2 and the values for the 1600-, 848-, and 748-cm.⁻¹ lines divided by the value for the 1750-cm.⁻¹ line to obtain ratios of absorbance related to per cent of undecomposed endrin. The use of an absorbance ratio instead of net absorbance eliminates instrumental and concentration variables from the data.

The variations of the net absorbance ratios for the 1600/1750, the 848/1750, and the 748/1750 peaks with per cent undecomposed endrin are shown in Figure 3. Any of these three ratios can be used to determine the decomposition of an endrin standard, but the 1600/1750 ratio is more sensitive in the high purity region and was selected for further study. An expanded graph covering 80 to 100% undecomposed endrin was used to improve estimation. Since the use of the absorbance ratio eliminates instrumental and concentration variables, such a curve can be used on any instrument. Values for reproduction are given in Table I.

To test the hypothesis that the use of the absorbance ratio eliminates instrumental variables, the spectra of potassium bromide disks of endrin standards A, B, and C were run on ten different infrared spectrometers. The data for standard A are shown in Table II. The data for the standard B disk showed an average of 88.2% undecomposed endrin with a range of 87.0 to 89.6% and a standard deviation of 0.9%, while those for standard C gave an average of 10.1% undecomposed endrin with a range of 9.5 to 12.0% and a standard deviation of 0.8%.

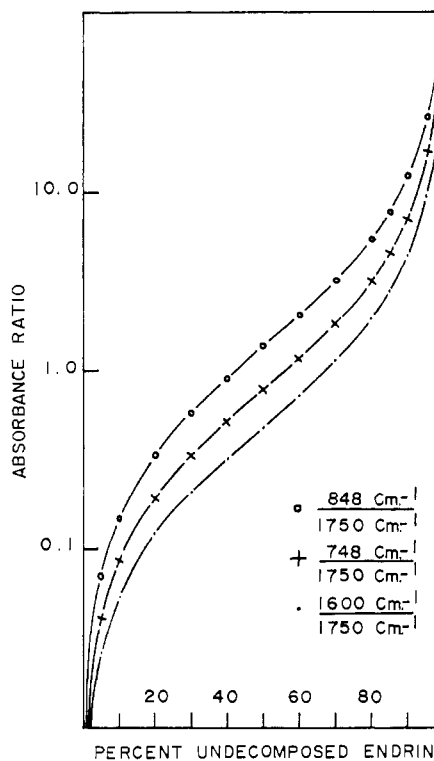


Figure 3. Variation of absorbance ratios with per cent undecomposed endrin

Table I. Absorbance Ratios and Per Cent of Undecomposed Endrin

% Undecomposed Endrin	Absorbance Ratio, A_{1600}/A_{1750}
99.9	478.2
99.5	95.3
99.0	47.4
98.0	23.5
97.0	15.5
96.0	11.5
95.0	9.10
94.0	7.50
92.0	5.51
90.0	4.31
88.0	3.51
86.0	2.94
80.0	1.92
60.0	0.718
40.0	0.319
20.0	0.120
10.0	0.053
5.0	0.025

Replication data for an individual disk of standard A for which the spectrum was determined periodically over a period of 14 days are shown in Table III and data for several disks of differing concentrations of standard B are shown in Table IV.

Disks of standard A subjected to various temperatures for 15 minutes indicated that decomposition is relatively slow until approximately 190° C. is reached. Above this temperature, the rate increases rapidly with temperature

Table II. Replication of Single Disk on Different Photometers

Instrument	A_{1600}	A_{1750}	Ratio	% Undecomposed Endrin
			A_{1600}/A_{1750}	
Beckman IR-5 ^a	0.189	0.009	21.0	97.8
Beckman IR-10 ^b	0.255	0.011	23.2	98.0
Beckman IR-12 ^b	0.198	0.012	16.5	97.2
Perkin-Elmer 21 ^b	0.148	0.007	21.1	97.8
Perkin-Elmer 225 ^b	0.259	0.009	28.8	98.4
Perkin-Elmer 257 ^b	0.240	0.010	24.0	98.1
Perkin-Elmer 337 ^b	0.296	0.010	29.6	98.4
Perkin-Elmer 457 ^b	0.293	0.008	36.6	98.7
Perkin-Elmer 700 ^b	0.198	0.009	22.0	97.8
Perkin-Elmer 337 ^c	0.310	0.011	28.2	98.4
Average			25.1	98.1
Standard deviation ^c			6.6	0.5

^a Instrument located at Dallas, Tex.^b Instrument located at Georgetown University, Washington, D.C.^c Calculated from range (Dean and Dixon, 1951).**Table III. Replication of Single Disk on Same Instrument^a**

Date	A_{1600}	A_{1750}	Ratio	% Undecomposed Endrin
			A_{1600}/A_{1750}	
April 3, 1968	0.310	0.016	19.4	97.6
April 4, 1968	0.317	0.014	22.6	97.9
April 5, 1968	0.302	0.012	25.2	98.2
April 8, 1968	0.309	0.012	25.8	98.2
April 9, 1968	0.309	0.012	25.8	98.2
April 16, 1968	0.310	0.015	20.7	97.8
Average			23.3	98.0
Standard deviation ^b			2.6	0.2

^a Perkin-Elmer 337 located at Dallas, Tex.^b Calculated from range (Dean and Dixon, 1951).**Table IV. Concentration Effect**

Concentration (% W./W.), Endrin/KBr	A_{1600}	A_{1750}	Ratio	% Undecomposed Endrin
			A_{1600}/A_{1750}	
0.25	0.142	0.045	3.16	86.8
0.39	0.217	0.062	3.50	88.0
0.52	0.255	0.077	3.31	87.4
0.63	0.321	0.093	3.45	87.9
0.76	0.360	0.114	3.16	86.8
Average			3.32	87.4
Standard deviation ^a				0.5

^a Calculated from range (Dean and Dixon, 1951).

and above approximately 215° C., endrin is essentially completely decomposed in 15 minutes. Analysis by gas-liquid chromatography of a sample heated for one hour at 195° C. showed approximately 1% of undecomposed endrin to be present.

The disk of standard A which was kept in an incubator at 35° C. and for which the infrared spectra were determined periodically showed an increase in decomposition from 99.1 to 93.8% undecomposed endrin in 12 months. The data taken at various times are shown in Table V.

GAS-LIQUID CHROMATOGRAPHY

The purities of standards B and C were also determined by gas-liquid chromatography on two different

Table V. Decomposition of Endrin with Time at 35° C.

Days	Absorbance Ratio	% Undecomposed Endrin
	Zero	49.0
13	25.7	98.2
20	22.9	98.0
27	20.0	97.7
40	17.9	97.4
109	15.8	97.0
129	15.0	96.9
165	13.0	96.4
200	11.2	95.9
236	8.71	94.8
368	7.24	93.8

columns and instruments using standard A as a reference. The chromatograms of all three standards on the Barber-Colman Model 5000 using the 3% XE-60 column are shown in Figure 4. The first peak (retention time 1.8 minutes) represents undecomposed endrin, while the second (retention time 4.6 minutes) is the aldehyde decomposition isomer, and the third (retention time 6.9 minutes) is the ketone decomposition product. Standard A shows no apparent peaks for either the aldehyde or ketone, standard B shows a small peak due to ketone but apparently none for aldehyde, and standard C shows peaks for both aldehyde and ketone. The ratio of aldehyde to ketone in standard C is approximately 15 to 85 (0.18), while gas-liquid chromatography on some columns which cause thermal decomposition during chromatographic separation gives aldehyde and ketone peaks of almost equal size (Phillips *et al.*, 1962). Calculation of the per cent of undecomposed endrin in standards B and C assuming standard A is 98.2% undecomposed endrin gives values of 88.5% for B and 16.0% for C on the Barber-Colman Model 5000 with the XE-60 column. Corresponding values obtained from the Barber Colman Model 10 with the DC-200 column are 90.0% for B and 12.8% for C. These values agree satisfactorily with those obtained by the proposed method.

DISCUSSION

The appearance of the absorption band at 1750 cm^{-1} due to the carbonyl stretch band of the ketone on the five-membered ring is the most sensitive method of detecting decomposition. The extent of decomposition may be estimated from the increase in the height of this band and may also be followed by the decrease in the band at 1600 cm^{-1} due to the chlorinated double bond of endrin or by the decrease in the characteristic endrin peaks at 848 and 748 cm^{-1} . Since the increasing absorbance, with decomposition, at 1750 cm^{-1} is a measure of the ketone isomer formed and the decreasing absorbance at 1600 cm^{-1} is a function of the undecomposed endrin remaining, the ratio between them should be a better measure of decomposition than either alone. The use of the absorbance ratio method also has the advantage of being essentially independent of the thickness or concentration of the potassium bromide

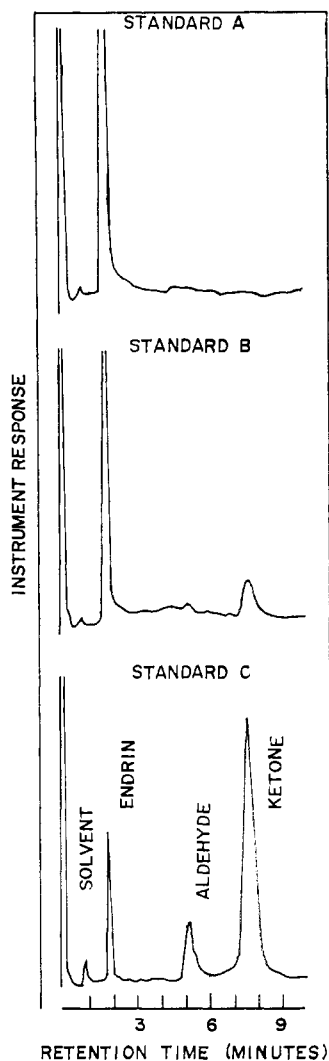


Figure 4. Gas-liquid chromatograms of endrin standards

disk and of instrumental parameters such as sensitivity. In addition, only 1 or 2 mg. of reference standard material is required and the method is extremely rapid. One hour is normally sufficient to complete the determination.

The sensitivity of the method indicates that 0.5% or less decomposition should be detectable and determinable in standards by using disk concentrations of approximately 1.3% (w./w.).

Heat-induced decomposition of endrin in KBr and in the open apparently follows the same route regardless of temperature to give a large excess of the ketone isomer over the aldehyde isomer. For this reason a badly decomposed standard will have an infrared spectrum essentially the same as that of the ketone isomer. The predominance of the ketone isomer is also shown in the gas-liquid chromatograms of standards B and C on the 3% XE-60 column. Standard B (88% undecomposed endrin) shows a small ketone peak and essentially no aldehyde peak while standard C (10% undecomposed endrin) shows almost six times as much ketone as aldehyde.

The effect of the KBr on the decomposition was checked by heating powdered standard A and a KBr disk of standard A at the same temperature for the same length of time. A disk was then made from the powdered standard. The infrared spectra of the two samples were essentially identical and essentially the same as that of the ketone isomer.

During gas-liquid chromatography on some columns at elevated temperatures, endrin decomposes to form approximately equal amounts of the ketone and aldehyde isomers (Phillips *et al.*, 1962). Recent work (MacDonell and Eaton, 1968) indicates that decomposition due to adsorption on and/or catalytic activity of the support medium may be reduced or eliminated by the use of surface-textured glass beads as the support medium.

Since endrin may be decomposed by both heat and light, standards should be stored in opaque bottles at below room temperature. The effect of ordinary storage conditions at room temperature as opposed to the suggested conditions is demonstrated by the purity of standards A and D. Even though these are samples of the same standard, A contains 98.2% undecomposed endrin while D contains 96.6%. Another example of the effect of storage conditions is the decomposition which occurred in the disk kept at 35° C. for several months. Even under these mild conditions, the amount of undecomposed endrin decreased significantly. As a consequence, standards should be evaluated upon receipt and stored under the recommended conditions.

Several commercial standards which were received during 1968 were evaluated by the proposed method and found to contain 93.0, 98.1, and 98.9% undecomposed endrin even though all were listed as being better than 99% pure.

The absorbance ratio technique used in this method should be extremely useful in the solution of similar problems in other areas utilizing infrared absorption.

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